Modulation of the hypothalamic–pituitary–adrenal axis of an Arctic-breeding polygynandrous songbird, the Smith's longspur, *Calcarius pictus*

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To successfully reproduce in the Arctic, birds must modulate their neuroendocrine and behavioural systems. These adjustments include an attenuation of the stress responsiveness of the hypothalamic–pituitary–adrenal (HPA) axis to external stimuli and a behavioural insensitivity to high corticosterone (B) levels. The HPA axis was examined in free-living territorial polygynandrous Smith's longspurs (*Calcarius pictus*) that migrate to breed on the Arctic tundra. Basal and stress-induced B levels were measured through the breeding season and were found to be significantly lower in females compared with males. This was not a consequence of adrenal insensitivity, because intrajugular injections of adrenocorticotrophin hormone (ACTH) enhanced B release in incubating females. In males the adrenocortical response to stress was significantly attenuated during the parental phase compared with arrival at the breeding ground. In contrast to temperate passerines, there was no significant decrease in male territorial aggressive behaviour when B was experimentally elevated, suggesting a behavioural insensitivity to glucocorticoids. This mechanism is hypothesized to increase reproductive success by preventing interruptions to parental care during transient deleterious environmental perturbations, which are often experienced in the short Arctic breeding season. Modulation of the HPA axis in this species in relation to life-history stage, lifetime reproductive success and the polygynandrous mating system is discussed.

Keywords: Arctic; aggression; birds; corticosterone; polygynandry; stress

1. INTRODUCTION

Breeding at high latitudes requires an ability to cope with potentially stressful conditions such as freezing temperatures, snowstorms and high winds. These events are unpredictable in the Arctic spring and summer, but when they occur they are generally short lived (e.g. Wingfield & Hunt 2002). Most Arctic breeding birds migrate thousands of kilometres to reach their breeding grounds and must nest shortly after arrival owing to the brevity of the breeding season (Hahn *et al.* 1995; Wingfield & Hunt 2002). Successful renesting is unusual, and most high latitude breeding birds typically have just one clutch a year (Hahn *et al.* 1995). Smith's longspurs (*Calcarius pictus*) are Arctic specialists. They find a mate and initiate egg laying within 4 days of arrival (S. L. Meddle, unpublished data). By contrast, birds breeding in temperate zones have a protracted breeding season that may last six months with many opportunities to nest (Wingfield & Farner 1993).

The erratic Arctic weather has the potential to induce an endocrine stress response that inhibits reproduction (e.g. Sapolsky 1993). The endocrine response to stressful conditions includes a rapid elevation in circulating glucocorticoids (Holmes & Phillips 1976; Wingfield & Farner 1993). This acute release of the glucocorticoid, corticosterone (B), from the adrenals is associated with significant increases in gluconeogenesis, locomotor activity and

varying effects on foraging behaviour (Wingfield & Silverin 1986; Gray *et al.* 1990; Astheimer *et al.* 1992; Rogers *et al.* 1993; Wingfield *et al.* 1998) as well as a decrease in aggressive, reproductive and parental behaviour (Silverin 1986; Wingfield & Silverin 1986). These behavioural changes may be adaptive because they facilitate transition back from this emergency response to normal activity (Wingfield *et al.* 1998). The magnitude of B secretion is dependent upon the nature of the stressor and the environmental or physiological context in which it is perceived. In other words, glucocorticoid secretion is modulated so that under certain circumstances there is a rapid reaction to a particular stressor whereas at other times there is an attenuated response (Wingfield 1994).

If during the short Arctic nesting season severe weather elicits a large adrenocortical response, then its effect would be the interruption or inhibition of reproduction. Thus to avoid the potentially disruptive effects of inclement weather and to maximize reproductive success, it is predicted that the stress response should be attenuated in Arctic breeding bird species. To investigate adrenocortical response modulations to stress between species, sexes, latitude of breeding site and different life-history stages, a capture, handling and restraint stress protocol is used. It reliably elicits an increase in B and has been routinely used for the past 20 years in wild birds (see Wingfield *et al.* 1982, 1992, 1995; Wingfield 1994).

As predicted, evidence for decreased adrenocortical responses to stress are found in Arctic breeding birds such as the Lapland longspur (*Calcarius lapponicus*), snow bunting (*Plectrophenax nivalis*), redpoll (*Carduelis flammea*),

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white-crowned sparrow (*Zonotrichia leucophrys gambelii*), savannah sparrow (*Passerculus sandwichensis*) and American tree sparrow (*Spizella arborea*) (Wingfield *et al.* 1994*a,b*, 1995; Romero *et al.* 1997, 1998*a*; Wingfield & Hunt 2002). Examples of stress modulation can also be found within a species, such as in the pied flycatcher (*Ficedula hypoleuca*), willow warbler (*Phylloscopus trochilus*), redpoll, savannah sparrow and snow bunting. Populations breeding at high latitudes all have lower maximum B levels induced by the capture stress protocol compared with populations breeding at low latitudes (Wingfield *et al.* 1994*a,b*; Silverin *et al.* 1997; Silverin & Wingfield 1998; J. C. Wingfield, unpublished data).

In addition to adrenocortical stress modulation, Arctic breeding birds have been shown to be insensitive to the behavioural effects of high B while metabolic effects remain. Territorial behaviour is maintained in American tree sparrows that have artificially elevated B levels (Astheimer *et al.* 2000). On the contrary, temperate breeding sparrows such as the song sparrow (*Melospiza melodia*) are behaviourally sensitive to B. Males show significant reductions in territoriality after experimental elevation of B (Wingfield & Silverin 1986).

All these examples have come from socially monogamous birds with both sexes sharing parental care of a single nest. By contrast the Smith's longspur is polygynandrous, meaning that females pair and mate with more than one male on a territory and at the same time males pair and mate with more than one female (Briskie 1992). Both sexes are highly territorial during the female fertile period (S. L. Meddle, unpublished data). Although this type of mating system has only been reported in a few avian species such as the dunnock, *Prunella modularis* (Davis 1985), and alpine accentor, *Prunella collaris* (Nakamura 1990), it ensures greater parental care for the chicks and the female encourages male parental care by copulating with several males. In addition, polygynandry allows males to have offspring in several different nests so that if one nest is lost, viable offspring remain in other nests.

Previous results show that the degree of parental care is reflected by maximum B levels reached by the capture handling protocol, with lower B observed in the sex that provides most parental care (Wingfield *et al.* 1995; O'Reilly & Wingfield 2001). As both sexes provide parental care in Smith's longspurs, we predict that there should be no sex difference in the adrenocortical response to stress once paternity is established.

The Lapland longspur is a related Arctic species that breeds in the same region, preferring tussock tundra to the damp grassland frequented by Smith's longspurs. The stress response of this socially monogamous longspur is seasonally modulated (Romero *et al.* 1998*a*), with no sex difference in the response (Wingfield *et al.* 1994*b*). This is also true for another closely related species, the snow bunting, which attenuates its stress response during moult (Wingfield *et al.* 1994*b*; Romero *et al.* 1998*a,b*). Neither species shows any significant decrease in territorial aggression after B implants (Meddle *et al.* 2001; J. C. Wingfield, unpublished data); indeed, parental care is unaffected in Lapland longspurs by this treatment (Hunt 1997).

The aim of this study was to examine the adaptations of the hypothalamic–pituitary–adrenal (HPA) axis of a polygynandrous Arctic specialist, the Smith's longspur. There were four main objectives: (i) define basal B levels through the short breeding season; (ii) determine the adrenocortical response to stress for both sexes; (iii) test adrenal gland sensitivity to adrenocorticotrophin-hormone (ACTH) to elucidate where HPA axis stress modulation occurs; and (iv) determine whether reproductive behaviour is sensitive to environmental stressors by experimentally elevating plasma B levels.

2. MATERIAL AND METHODS

(a) Subjects

Free-living Smith's longspurs were captured on their breeding territories in May and June from 1996 to 2001 in the vicinity of the Toolik Field Station (University of Alaska, Fairbanks, Institute of Arctic Biology, 68°38′ N, 149°38′ W). Breeding populations were restricted to a 1 km band either side of the Atigun and Sagavaninktok rivers. Birds were caught in mist nets (Avinet Inc. Dryden, NY, USA) and banded for subsequent identification with US Fish and Wildlife Service bands and unique colour band combinations. Smith's longspur breeding plumage is sexually dimorphic allowing easy gender identification in the field. All experimental procedures were done in accordance with guidelines defined by the Institutional Animal Care and Use Committee at the University of Washington.

(b) Body measurements

Body measurements were taken from all birds. They were weighed (in grams), scored for fat deposits (average of furcular and abdominal fat measured on a semi-quantitative scale of 1 to 5 with 5 being the fattest (Helms & Drury 1960)) and assessed for moult, as indicated by primary feather loss and regrowth. Male plumage and the presence of a cloacal protuberance identified males and brood patches were scored in females.

(c) Changes in basal corticosterone through the breeding season

Basal B levels were measured throughout all stages of the breeding cycle. The breeding season was divided into five phases determined by behavioural observations and physiological measurements: (i) arrival on breeding grounds; (ii) pair formation; (iii) mate guarding during egg lay; (iv) incubation; and (v) feeding nestlings, including the start of moult. Immediately on capture (within 3 min) a 100 μl blood sample was taken for basal B measurement by puncturing the alar wing vein with a 25 G needle and collecting the blood into heparinized microhaematocrit tubes. Blood was kept on ice until it was centrifuged. The plasma was stored at −20 °C until transported frozen to the University of Washington.

(d) Adrenocortical response to capture stress

Assessment of the adrenocortical response to capture and restraint stress was performed as follows. Both males (*n* = 9) and females (*n* = 8) were caught through pair formation to incubation phases and males (*n* = 9) were captured later on in the season while they were feeding nestlings. Within 3 min of capture a basal blood sample was taken and subsequent samples were taken at 5, 10, 30 and 60 min. Blood samples were kept on ice until processed as outlined above and assayed for B. Between

sampling the birds were held in opaque cloth bags and protected from adverse weather conditions. Body measurements were taken (as outlined above) between the 10 and 30 min samples.

(e) Adrenal response to adrenocorticotrophin hormone

To test adrenal responsiveness, ACTH was administered to female longspurs during incubation. Within 3 min of capture a 100 µl blood sample was taken and immediately the bird received an intrajugular injection of 100 IU kg^{-1} ACTH dissolved in lactated Ringers solution ($n = 6$) (Baxter Healthcare Co., Deerfield, IL, USA) using a 30 G needle. Injection of Ringers served as a control ($n = 3$). Aliquots of ACTH and Ringers solution were kept frozen and defrosted immediately before use. Repeated blood samples were taken at 5, 10, 30 and 60 min and were kept on ice and processed for B as outlined above. During sampling, body measurements were taken and the birds were held and protected in opaque cloth bags.

(f) Behavioural response to corticosterone treatment

Territorial male Smith's longspurs were captured during the early mate guarding phase and a 100 µl blood sample was immediately taken for B measurement. Less than 5 min later they were implanted with either an empty ($n = 12$) or B (Sigma, St Louis, MO, USA) filled ($n = 10$) implant. The B was packed into a 16 mm length of silastic tubing (1.47 mm inner and 1.96 mm outer diameter; Dow Corning Midland, Michigan, IL, USA) sealed at each end with silastic sealant. Before implantation one end was cut off to facilitate B diffusion from the implant (Wingfield & Silverin 1986). Each male received a single implant placed subcutaneously via a small flank incision sealed with Nexaband (Veterinary Products Laboratories, Phoenix, AZ, USA). Measurable amounts of B are released within hours and last for at least a week (see, for example, Wingfield & Silverin 1986). Body measurements were taken and the birds were released within 7 min of capture.

(g) Simulated territorial intrusion

To assess behavioural effects of B on territorial behaviour, birds were subjected to a simulated territorial intrusion (STI) 48 to 96 h after implantation. A caged male Smith's longspur decoy was positioned within the focal bird's territory. Song was broadcast through a battery operated guitar loudspeaker placed alongside the decoy cage. The song sequence was repeated every 10 to 15 s (randomized from three Smith's longspur males recorded in previous years). The audible range of the speaker was at least 100 m. This standard 'challenge' protocol has been used extensively to assess aggressive behaviour in passerines (see, for example, Wingfield & Ramenofsky 1985). During the 15 min challenge, the number of songs, short flights, closest approach by the male to the decoy and the time spent within 5 m of the decoy were recorded. Individuals that did not respond to the STI were assigned a closest approach of 100 m. The behavioural response to an STI from unimplanted males on the same territories as the implanted birds was recorded at the same time ($n = 6$).

All STIs were conducted within 3 days during the mate guarding phase between the hours of 0830 and 1800 Alaska Standard Time. No STIs were undertaken during inclement weather. During behavioural recordings the observer was blind to the bird's treatment and directly afterwards the birds were recaptured and a 100 µl blood sample and body measurements were

taken and the implant removed. Evidence of a physiological effect of B was made by judgement of pectoral muscle condition by noting the extent of curvature around the sternal keel.

(h) Corticosterone assay

B concentration was measured in duplicate for each plasma sample by radioimmunoassay after extraction of 10 µl samples in 4 ml of redistilled dichloromethane. Extracts were re-dissolved in 0.1 M phosphate buffer. Inter-assay variation was estimated by the inclusion of B standards. Water-only blanks were below the detection limits in all cases. The average inter-assay and intra-assay variation determined by standards and plasma pools for the assays run in this study was less than 14% and 6%, respectively. Recoveries after extraction ranged between 70% and 94% (measured for each sample independently and adjustments made to the final measured level accordingly). Fifty per cent binding was *ca.* 100 ± 8 ng ml^{-1}. For full details see Wingfield *et al.* (1992).

(i) Statistical analysis

All data are presented as means ± s.e.m. The behavioural, body measurements and hormonal data through the breeding season were analysed with a one-way or a two-way repeated measures analysis of variance (ANOVA) for normally distributed data or a Kruskal–Wallis one-way ANOVA on ranks for data failing the normality test. Pairwise multiple comparisons procedure (Dunn's method) were performed to isolate groups that differ from others. Body weight and fat were compared between treatments by using non-parametric Mann–Whitney U-tests. Significance was set at an alpha level of $p < 0.05$.

3. RESULTS

(a) Changes in basal corticosterone and body measurements through the breeding season

Basal B levels did not vary significantly through the breeding season in females ($H = 1.878$, $p = 0.598$, one-way ANOVA). Basal levels were low ranging from 4.0 ± 0.64 ng ml^{-1} during incubation to 7.22 ± 1.77 ng ml^{-1} at pair formation (figure 1). In males, basal B changed through the breeding season with levels during the parental phase significantly lower (2.05 ± 0.29 ng ml^{-1}) than arrival (8.96 ± 1.24 ng ml^{-1}), pair formation (15.74 ± 3.26 ng ml^{-1}) or mate guarding (9.75 ± 0.89 ng ml^{-1}, $H = 23.12$, $p < 0.001$). For the entire period between arrival and incubation, females had significantly lower basal B levels (5.33 ± 0.77 ng ml^{-1}) compared with males (11.05 ± 1.12 ng ml^{-1}, $p < 0.001$, Mann–Whitney rank sum test).

In males there was no significant change in body mass through the breeding season (season effect: $F_{4,108} = 1.891$, $p = 0.117$). However, female body mass (season effect: $F_{4,108} = 3.536$, $p < 0.05$), was significantly higher during egg lay (29.8 ± 0.9 g, $n = 6$) compared with arrival (26.1 ± 0.4 g, $n = 8$; $p < 0.05$). There was no significant difference in body mass between males (27.6 ± 0.1, $n = 104$) and females (27.9 ± 0.3, $n = 50$) when averaged from arrival through incubation ($p = 0.324$). Fat score did not significantly vary through the breeding season for either males ($p = 0.143$) or females ($p = 0.204$). None the less, females (2.5 ± 0.09, $n = 50$) had on average a significantly higher fat score compared with males (0.9 ± 0.05, $n = 104$, $p < 0.001$). None of the birds from arrival
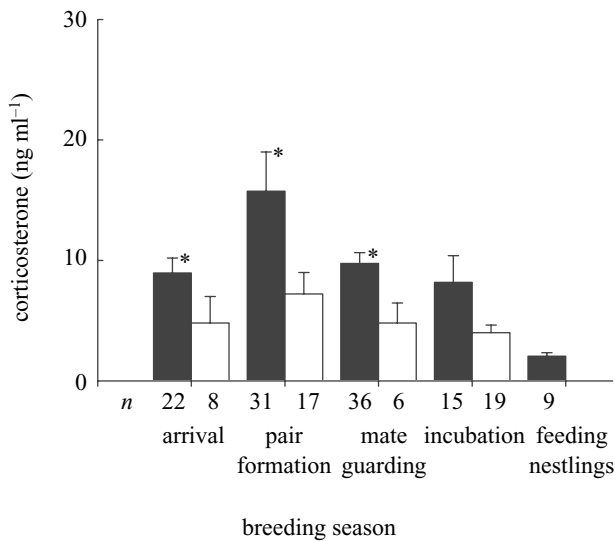
Figure 1. Sex differences in baseline B levels in Smith's longspurs through the breeding season. Each bar represents the mean + s.e.m. for the sample size indicated under each bar (filled bars, males; open bars, females). There is no significant difference in baseline B for females across the breeding season ($p > 0.1$). For males, baseline B is significantly lower during the feeding of the nestlings compared with arrival, pair formation and mate guarding ($* p < 0.05$). From arrival through incubation, plasma B levels in females are significantly lower than in males ($p < 0.001$).

through incubation was undergoing feather moult. During the feeding of the nestlings three out of nine males were moulting primary feathers. There was no significant difference in basal B levels between non-moulting (2.10 ± 0.43) and moulting birds (1.95 ± 0.27; $t = 0.23$; $p = 0.827$).

(b) Adrenocortical response to capture stress

Capture handling stress elevated B in male longspurs during the first 60 min of capture (time effect: $F_{4,63} = 43.53$, $p < 0.001$; figure 2). In males, the magnitude of the B response to capture handling stress was significantly attenuated during nestling feeding as compared to early breeding season (time of breeding season effect: $F_{1,63} = 59.26$, $p < 0.001$; figure 2). The temporal dynamics of B concentration were also significantly different between the time of breeding season (breeding season × time of sampling interaction term: $F_{4,63} = 23.57$, $p < 0.001$; figure 2). Maximum B levels reached 85.0 ± 10.0 ng ml^{-1} during the early breeding phase ($n = 9$) compared with 15.22 ± 2.55 ng ml^{-1} during the parental phase ($n = 9$). Capture handling stress also significantly elevated B in female longspurs during the first 60 min of capture (time effect: $F_{4,63} = 46.78$; $p < 0.001$; figure 2). In addition, there was a significant sex difference in B levels reached in response to capture handling during the early breeding phase (sex effect: $F_{1,60} = 26.27$; $p < 0.001$; figure 2). Female maximum B levels were 26.37 ± 2.48 ng ml^{-1}, ca. 75% less than males during the early breeding stage. The temporal dynamics of B concentration also significantly differed between the sexes (sex × time of sampling interaction term: $F_{4,63} = 16.42$, $p < 0.001$; figure 2).
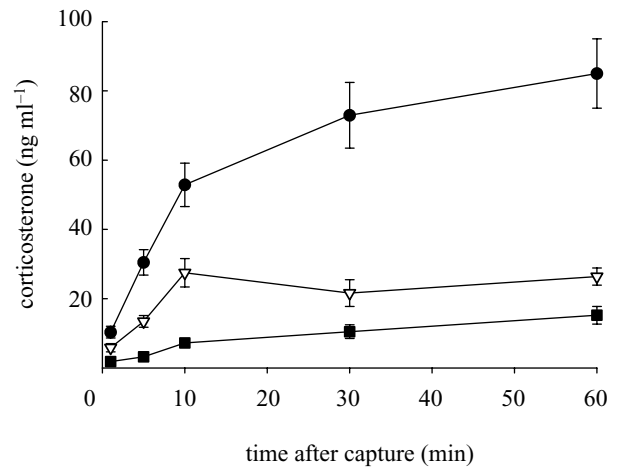
Figure 2. Changes in plasma B levels (± s.e.m.) during capture stress of male ($n = 9$) (circles) and female ($n = 8$) (triangles) Smith's longspurs during the early breeding phase and males during the parental phase ($n = 9$) (squares). The response to stress is significantly attenuated in males during the parental phase as compared to the early breeding phase ($p < 0.001$). There is a significant sex difference in the stress response in the early breeding phase, with males having a greater response compared with females ($p < 0.001$).
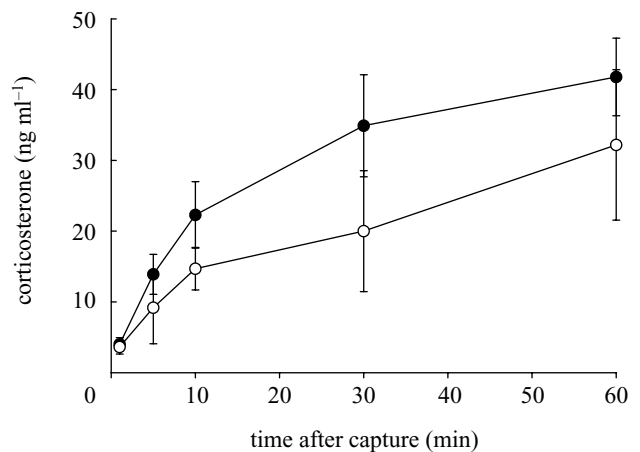

Figure 3. Changes in plasma B levels (mean ± s.e.m. over a 1 h period) during capture stress of incubating female Smith's longspurs following intrajugular injection of 100 IU kg^{-1} ACTH ($n = 6$) (filled circles) compared with Ringer's control ($n = 3$) (open circles). ACTH significantly increased B secretion compared with controls ($p < 0.05$).

(c) Adrenal response to adrenocorticotrophin hormone

Capture handling stress and intrajugular injections into female longspurs significantly elevated B during the first 60 min of capture (time effect: $F_{4,21} = 10.64$, $p < 0.001$; figure 2). Intrajugular injections of ACTH into females significantly elevated stress-induced B levels compared with controls over a 1 h period (treatment effect: $F_{1,21} = 6.85$, $p = 0.033$; figure 3). Maximum stress-induced B levels reached 32.2 ± 10.63 ng ml^{-1} ($n = 3$) in controls compared with 41.78 ± 5.47 ng ml^{-1} ($n = 6$) in ACTH treated birds. The temporal dynamics of B concentration did not significantly differ between treatments
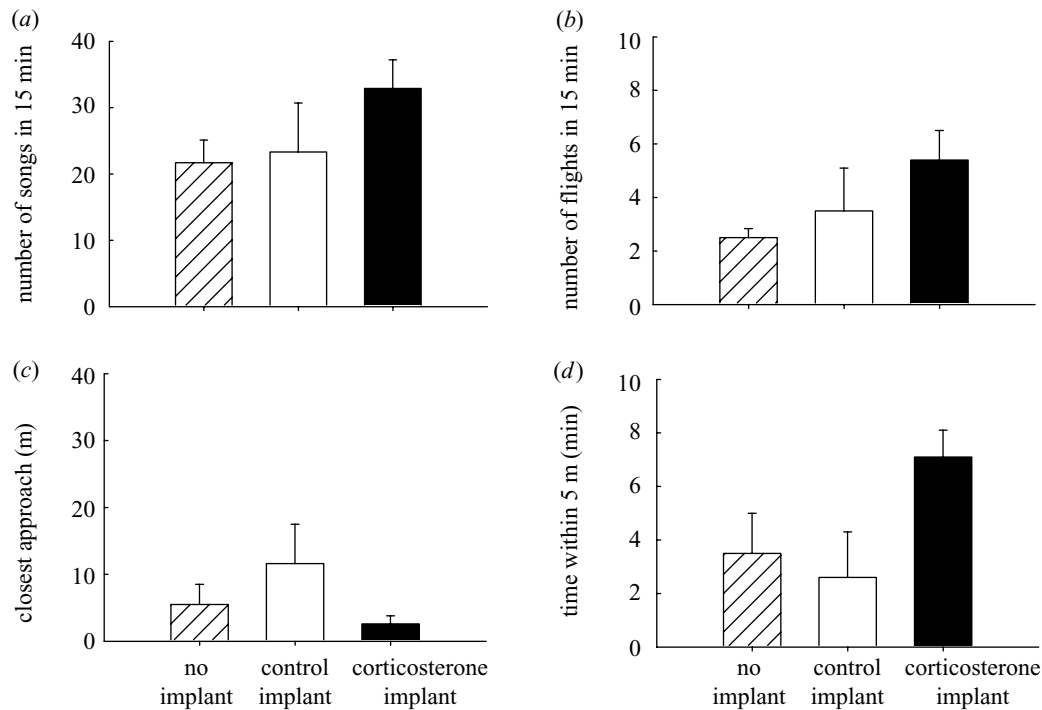
Figure 4. Behavioural responses of control ($n = 8$) or B ($n = 8$) implanted and unimplanted ($n = 6$) male Smith's longspurs to a 15 min STI. The number of (*a*) songs, (*b*) flights, (*c*) closest approach and (*d*) time the focal bird spent within 5 m of the decoy cage are presented. Bars are means + s.e.m.; $p > 0.1$.

(treatment × time of sampling interaction term: $F_{4,21} = 0.609$, $p = 0.66$; figure 2).

(*d*) *Effects of corticosterone treatment on behavioural responses to simulated territorial intrusion*

B treatment had no effect on territorial behaviour (figure 4). The number of songs ($F_{2,19} = 1.17$, $p = 0.33$) and flights ($F_{2,19} = 1.41$, $p = 0.27$), closest approach to the decoy ($H = 2.60$, $p = 0.27$) and time spent within 5 m of the decoy in response to STI ($F_{2,19} = 3.07$, $p = 0.07$) did not significantly differ between unimplanted ($n = 6$), control ($n = 8$) and B implanted birds ($n = 8$). B implants increased basal B to physiologically relevant elevated levels (26.43 ± 0.29 ng ml^{-1}, $n = 3$), whereas levels in control implants (7.35 ± 0.99 ng ml^{-1}, $n = 2$) were in the range of those observed in unimplanted birds (see figure 1). Evidence for the catabolic effect of the B implant was observed by pectoral muscle shrinkage and fat deposition in implanted birds (fat score 1.2 ± 0.15 versus 0.75 ± 0.25 in controls). Muscle protein breakdown is brought about by gluconeogenesis, the known effect of B (e.g. Chester-Jones *et al.* 1972). When the implant was recovered from the bird *ca.* 75% of B remained.

4. DISCUSSION

(*a*) *Seasonal modulation of the adrenocortical response to stress*

These findings in Smith's longspurs support mounting evidence that many bird species breeding in severe environments alter their neuroendocrine system to suppress or at least reduce their adrenocortical responses to environmental perturbations (Wingfield *et al.* 1994*a*,*b*;

Silverin *et al.* 1997; Silverin & Wingfield 1998; Lynn 2002; Wingfield & Hunt 2002).

More importantly, we have provided evidence to suggest that, for males at least, the stage of breeding plays a part in determining the adrenocortical stress response, as the HPA axis on arrival is more sensitive to acute stress than after paternity has been determined. Basal B levels are highest during the early part of the breeding season while the males are establishing territories and intensively defending their paternity, and this may well reflect the stress of this time period. It is consistent with predictions of Wingfield *et al.* (1995) that males should attenuate their stress response during nestling feeding, and such modulations have been previously observed in male white-crowned sparrows and pied flycatchers (see Silverin 1998*a*; Romero & Wingfield 1999).

Parental care is the most energetically demanding period during the breeding season and may provide another explanation for acute stress modulation at this time (e.g. Astheimer *et al.* 1995; Silverin 1998*a*). Moult occurs simultaneously with parental care in this species (S. L. Meddle, unpublished data) and a reduction in catabolic processes by lowering circulating B levels and attenuating the adrenocortical stress response allows energy to be directed both into feather growth and parental duties. This phenomenon is widespread, with the lowest baseline B and adrenocortical stress responses reported during moult in the Lapland longspur (Astheimer *et al.* 1995; Romero *et al.* 1998*a*), snow bunting (Romero *et al.* 1998*b*), willow tit, *Parus montanus* (Silverin 1997) and white-crowned sparrow (Wingfield *et al.* 1982; Astheimer *et al.* 1994; Romero & Wingfield 1999).

The observed sex difference in the response to acute stress in Smith's longspurs early in the breeding season is

novel and may be related to their polygynandrous mating system. So far, none of the closely related Arctic species such as the Lapland longspur, snow bunting or the mid-latitude breeding McCown's (*Calcarius mccownii*) and chestnut collared (*Calcarius ornatus*) longspur, shows such a sex difference in the adrenocortical stress response. However, these species are socially monogamous (Wingfield *et al.* 1994*b*; Astheimer *et al.* 1995; Lynn 2002). In the socially monogamous Gambel's white-crowned sparrow there is no sex difference observed in the stress response during the parental phase. None the less, early in the breeding season and during migration i.e. before any parental investment, there is a higher response in males compared with females (Wingfield *et al.* 1982; Astheimer *et al.* 1994; Romero *et al.* 1997). This, as in Smith's longspurs, suggests bi-directional modulation in males: up in early breeding and then down in the parental phase. Together, these results lend support to the hypothesis that modulation of the adrenocortical response should occur when birds invest heavily in parental care, thus avoiding B-mediated escape behaviour (Wingfield *et al.* 1995; Silverin 1998*a*; O'Reilly & Wingfield 2001).

(b) *Adrenal response to adrenocorticotrophin hormone*

The attenuated B response to acute stress in females appears not to originate at the level of the adrenal gland as exogenous ACTH significantly increased B secretion. This implies that the modulation of the adrenocortical response to stress is occurring in the brain at the level of the pituitary or within the hypothalamus. None the less, B levels reached after ACTH administration are still *ca.* 50% less than those observed in males at the beginning of the breeding season. It is reported that male white-crowned sparrows on arrival on their Arctic breeding grounds exhibit increased levels of corticosteroid binding protein (CBP) (Romero *et al.* 1997). The amount of circulating CBP that renders B biologically inactive is known to vary seasonally, thus buffering any increased levels of B (Wingfield & Farner 1980; Silverin 1986; Romero *et al.* 1998*a*; Deviche *et al.* 2001). Further investigations are warranted to examine adrenal sensitivity at other times during the year, and whether there are dynamic changes in CBP levels through the breeding season in Smith's longspurs.

(c) *Behavioural insensitivity to corticosterone*

Decreases in territorial behaviour with artificially elevated B are observed mainly in mid-latitude birds breeding in temperate climates (see, for example, Cain & Lien 1985; Silverin 1986; Wingfield & Silverin 1986; Silverin 1998*a,b*; Wingfield *et al.* 1998). By contrast, it is advantageous for species with severe temporal constraints on their breeding schedules to be insensitive to the behavioural effects of high glucocorticoid levels, which would otherwise disrupt breeding activities. This behavioural insensitivity hypothesis is supported by our data from Smith's longspurs. The experimentally elevated levels of B had no effect on territorial behaviour, but did increase circulating plasma B and cause flight muscle wastage: a well-documented catabolic effect of glucocorticosteroids (see Chester-Jones *et al.* 1972; Harvey *et al.* 1984; Wingfield *et al.* 1998). Further support for a B insensitivity

hypothesis in Arctic breeding birds is provided by studies on the closely related species the Lapland longspur, with B implanted males showing no changes in parental care (Hunt 1997) or aggressive behaviour (J. C. Wingfield, unpublished data). Similarly in the American tree sparrow (Astheimer *et al.* 2000), snow bunting (Meddle *et al.* 2001), savannah sparrow (S. L. Meddle, unpublished data) and pied flycatcher (Silverin 1998*b*) B implants in free-living males had no significant effect on territorial aggression. Nevertheless the Arctic breeding Gambel's white-crowned sparrow does show a behavioural response to experimentally elevated B (Meddle *et al.* 2002). The reason for this anomaly may be explained by spatial opportunism. In contrast to Smith's longspurs, Gambel's white-crowned sparrows show low site fidelity and do not settle if their traditional breeding sites are covered in deep snow, i.e. they are not territorial if deleterious weather conditions increase their B levels (Hahn *et al.* 1995).

(d) *Conclusions and implications*

Although many Arctic-breeding birds modulate their adrenocortical response to acute stress, male Smith's longspurs do not appear to show any attenuation of their stress response early in the breeding season but they are insensitive to the behavioural effects of experimentally elevated B once their territories have been established. Taken together with similar findings in Gambel's white-crowned sparrows and pied flycatchers (Romero *et al.* 1997; Silverin 1998*a*), these results suggest an alternative strategy by some male bird species on arrival on their breeding grounds. During competition for paternity, the HPA axis in these species is more sensitive to acute stress. If conditions deteriorate they can leave, but once the parental phase of breeding is underway the stress response is modulated so that parental investment takes priority. Further experiments must be undertaken to find out when exactly this modulation occurs and what factors and neuroendocrine mechanism control it. As the polygynandrous mating system involves variable parental investment, this leads us to propose the hypothesis that individuals with potentially high degrees of reproductive success may suppress glucocorticoid responses whereas individuals with a low degree of reproductive success do not.

These findings raise the question that if males are not modulating their stress response why then are they behaviourally insensitive to elevated B levels? This may be explained in part by the abundance and activity of the glucocorticoid receptors and a seasonal variation in binding capacity of CBP. Studies have shown that there are seasonal changes in corticosteroid receptors in the brain of the house sparrow, *Passer domesticus* (Breuner & Orchinik 2001) and seasonal changes in CBP in passerines (Wingfield & Farner 1980; Silverin 1986; Romero *et al.* 1998*a*; Deviche *et al.* 2001). However, nothing is known at present about any seasonal change in CBP or brain glucocorticoreceptors in Smith's longspurs. Further research is required to investigate the underlying mechanisms regulating stress modulation and the behavioural B insensitivity in this polygynandrous Arctic breeding species.

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